

Briefing Document

Biological Response Modifiers Committee Meeting # 37

Cellular Products for the Treatment of Cardiac Disease

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INTRODUCTION

This Biological Response Modifiers Advisory Committee (BRMAC) is convened to provide the FDA with insight and perspectives regarding the major issues confronting the development of cellular products for the treatment of cardiac diseases. These issues include manufacturing, catheter-product interactions, the nature and quantity of preclinical data and concerns related to early phase clinical studies. Controversy surrounds the extent and nature of manufacturing information and preclinical data necessary to support the introduction of these cellular products into clinical studies. Because the majority of these cellular products are autologous, some investigators have cited them as inherently safe and have suggested that preclinical studies may be unnecessary. Some investigators have proposed initiation of phase 2 clinical studies without exploration of safety in phase 1 studies. Others have suggested that without a detailed understanding of the cellular products' characteristics and exploration of safety and mechanisms of action in preclinical studies, it is impossible to design and safely conduct clinical studies. Given these widely divergent opinions, FDA has convened this BRMAC to discuss the issues in a public forum.

No specific products are being presented for regulatory review at this meeting and no data presented at the meeting will have undergone FDA review for completeness or accuracy. Instead, published information will be presented and leading researchers in the field will present their viewpoints on the major issues confronting this area of research. Members of the BRMAC will be requested to consider these publications and view points and provide a response to FDA questions. While a consensus response to these questions is desirable, no consensus is required. Since the field is rapidly developing, FDA anticipates that all opinions are tentative and subject to reconsideration based upon accumulating data.

MEETING GOALS

This meeting is organized to achieve the following goals regarding the development of cellular products for the treatment of cardiac diseases:

- ?? Provide FDA with perspectives on the types of manufacturing and preclinical data critical to the initiation of clinical studies
- ?? Provide FDA with perspectives on the major issues in the design, conduct and analyses of exploratory clinical studies
- ?? Provide a public forum to discuss the major controversies in developing these products

BACKGROUND

General:

Despite many recent advances, ischemic heart disease and congestive heart failure (CHF) remain the major causes of morbidity and mortality in the USA. Despite the important advances in therapy of the last two decades, CHF continues to be a disease characterized by high morbidity and mortality. CHF because of its high prevalence (1-2% of the adult population in the U.S.A) and frequent requirement for hospitalization is among the most costly medical problems in the country. CHF continues to increase in prevalence because 1) the incidence is related to age and the average age of the American population is increasing and 2) reperfusion therapy has led to growing numbers of patients surviving acute myocardial infarction with diminished cardiac reserve.

Similarly, despite advances in medical therapy and percutaneous interventional techniques, ischemic heart disease remains a major cause of morbidity and mortality. A recent paper estimated that 100,000 to 200,000 patients per year develop coronary artery disease not amenable to conventional revascularization, either coronary artery bypass grafting (CABG) or percutaneous coronary intervention (PCI) (Mukherjee, Bhatt et al. 1999). Further, many more patients would benefit from revascularization techniques that are less invasive, more durable, and more complete.

Cellular therapies for cardiac disease are a burgeoning field of clinical research as potential treatments for patients with congestive heart failure and/or ischemic heart disease. This research to date has involved cells derived from autologous muscle biopsies, hematopoietic stem cells from autologous peripheral blood after mobilization, or mesenchymal or hematopoietic stem cells obtained from bone marrow. They have been/or are proposed to be administered through catheters into the coronary arteries, transendocardially through injection catheters into the left ventricular myocardium, or transepically through a needle during concomitant CABG.

Cellular products to be discussed at this meeting consist of the following:

- ?? Cellular products manufactured without *in vitro* culture methodology, a group that includes most peripheral blood and/or bone marrow-derived cells, and
- ?? Cellular products manufactured with *in vitro* culture methodology, a group that includes cells derived from skeletal muscle biopsies and certain types of bone marrow-derived cells.

In general, the cellular products to be discussed are administered by one of the following routes:

- ?? By transepically injection into the left ventricular myocardium during thoracotomy; in this procedure the cellular product is injected into the myocardium using a needle and syringe under direct visualization,

- ?? By transendocardial injection into the left ventricular myocardium via percutaneous catheterization; in this procedure the cellular product is injected using an investigational catheter which is passed retrograde through the aorta into the left ventricle; the investigational catheter contains a needle and once the catheter is placed against the left ventricular endocardium, the needle is extruded, and the cellular product injected into myocardium,

- ?? By injection through a catheter into the coronary artery lumen; the coronary artery lumen is occluded by a balloon and the cellular product infused into the distal coronary artery lumen

Discussions of peripheral blood and/or bone marrow-derived cells and cells derived from skeletal muscle biopsies will focus primarily on the use of autologous cellular products, because only autologous cells have been described in published clinical reports.

Citations to “stem cells” will occur frequently in this document. Bone marrow and growth factor mobilized peripheral blood have been widely described as containing stem cells, capable of regenerating and assuming phenotypic characteristics of a variety of tissues, including cardiac tissue. Consequently, in this document these cells will be referred to as “bone marrow stem cells” (BMSC) or “peripheral blood stem cells” (PBSC).

Cellular products derived from skeletal muscle biopsies are most commonly cited as consisting of differentiated skeletal muscle cells that are capable of regeneration. These cells are commonly referred to as “myoblasts” and are not usually cited as “stem cells.”

The reader is referred to the NIH document attached to this document for a glossary of the terms related to stem cells. Of note, stem cell products derived from human embryonic tissue are not a discussion focus for this meeting.

Regulatory:

FDA regulates cellular products for cardiac diseases as drugs and biological products. This regulatory paradigm is based, in part on manufacturing procedures, the use of investigational devices in some studies, the non-homologous use of the cellular products and safety concerns associated with administration of these products. Consequently, FDA requires Investigational New Drug Applications (IND) for cellular products being evaluated for the treatment of cardiac diseases.

The regulatory pathway for cellular products is an evolving process and certain issues related to the ultimate licensure of cellular products remain to be resolved. Hence, this meeting will focus solely upon the scientific basis for clinical development of cellular products to be used in the treatment of cardiac diseases. Conceivably, FDA may request future BRMAC meetings or other venues to discuss the regulatory issues associated with late-phase clinical development of these cellular products.

For ease of reference, questions to the BRMAC are cited within the text of this document and also are listed at the end of the document.

MANUFACTURING INFORMATION

Most investigational cellular products are intended to replace missing, damaged or diseased cells with cells that are healthy and functional. Attempts to develop a cellular product that can restore defective cardiac function with cells not derived from cardiac tissue assumes the presence of undifferentiated or partially differentiated cells that can develop into the appropriate cardiac cellular phenotype. These non-cardiac cells must be capable of facilitating a variety of activities not usually associated with their tissue of origin, such as revascularization, muscle regeneration and electrical conduction (Orlic, Hill et al. 2002). Certain *in vitro* studies have shown that non-cardiac cells may acquire functions characteristic of cardiac cells. For example, unfractionated bone marrow cells, which do not normally secrete measurable amounts of vascular endothelial growth factor (VEGF), can do so after 4 weeks in culture, indicating the existence of a cell population that may facilitate angiogenesis when introduced into myocardium (Fuchs, Satler et al. 2003).

Cellular products under investigation for cardiac repair fall into two broad categories based on processing and manufacturing procedures.

- ?? In the first group are cells administered to subjects immediately after collection or processing. This group consists of cellular products derived from bone marrow aspirates or peripheral blood cells after administration of G-CSF. These products contain stem cells, and investigators theorize that these undifferentiated bone marrow-derived progenitor cells can differentiate *in vivo* into angioblasts and/or cardiomyocytes (Orlic, Hill et al. 2002).

- ?? The second group consists of cells that are expanded and/or differentiated *ex vivo* by a multi-step manufacturing process involving an extended culture and incubation period prior to administration to subjects (Pagani, DerSimonian et al. 2003). This group includes cells derived from skeletal muscle biopsies and certain bone marrow mesenchymal cells.

Cellular Products Manufactured Without *in vitro* Culture Methodology

Almost all cellular products manufactured without *in vitro* culture methodology are derived from blood or bone marrow. Bone marrow is the source of the progenitor cells that have been associated with repair or regeneration of damaged myocardium in most preclinical studies. These cells are generally presumed to be similar to the human cellular product identified by expression of CD34, a surface glycoprophosphoprotein appearing on 2-4% of normal human bone marrow cells. A recently described cell surface antigen, CD133, is expressed on a subset of human CD34+ cells including immature myeloid and monocytic progenitors and this antigen is occasionally cited in the investigational literature (Wognum, Eaves et al. 2003). Large numbers of CD34+ cells can be collected directly from bone marrow aspirates or from growth factor-mobilized peripheral blood and can be induced to expand and differentiate into a variety of cell types when cultured with cytokines and growth factors (Gunsilius, Gastl et al. 2001). The cell number and phenotype of the blood and bone marrow-derived products vary, depending on the individual donor and on whether the cells are collected from bone marrow or growth-factor mobilized peripheral blood.

Cell Collection

Bone marrow is generally collected from the posterior iliac crest by multiple punctures with a hollow needle and syringe. The marrow is aspirated in 5-10 mL aliquots and expelled into a diluent containing an anticoagulant, usually heparin. A series of progressively finer filters removes bone spicules and clots from the collected marrow. PBSC products are collected by an apheresis procedure using a continuous flow cell separator and a citrate anticoagulant.

BMSC & PBSC Processing

Bone marrow is a heterogeneous mixture of hematopoietic stem cells and erythroid, myeloid, monocytic, lymphoid and thrombocytic cells at various stages of maturation. A few preclinical and clinical studies have examined the administration of bone marrow-derived cellular products obtained immediately after their collection and filtration, a process that does not involve fractionation of the cells into more specific phenotypes. However, most investigators have incorporated manufacturing procedures that use a post-filtration isolation procedure in which the diluted bone marrow is layered on a density gradient, centrifuged and washed multiple times (Strauer, Brehm et al. 2002; Perin, Dohmann et al. 2003). This procedure yields a distinct layer of light density mononuclear cells enriched in progenitor and stem cells that can be removed from the high density red blood cells and polymorphonuclear leukocytes. However, this enriched product still contains large numbers of other cell populations at various stages of development.

Growth factor-mobilized peripheral blood, like bone marrow, contains a variety of cell populations. The peripheral blood apheresis process separates cellular components by density, harvesting the mononuclear cells and reinfusing most of the platelets, red blood cells and neutrophils to the patient. The collected mononuclear cell component is made up primarily of lymphocytes, monocytes and CD34+ hematopoietic progenitors. Immunomagnetic systems are available for stem and progenitor cell selection using anti-CD34 antibody and paramagnetic microspheres. These selection systems can produce a PBSC product containing 70-90% CD34+ cells from a starting material of 1-3% CD34+ cells. This process provides a product enriched in the CD34+ cells hypothesized to participate in cardiac repair (Yeh, Zhang et al. 2003).

Cellular Products Manufactured With *in vitro* Culture Methodology

Other cellular products being studied for cardiac repair are those that undergo an *in vitro* culturing process before administration. Preclinical studies have been reported using cultured autologous skeletal myoblasts and autologous or allogeneic bone marrow-derived mesenchymal stem cells (MSC) (Orlic, Hill et al. 2002; Reffelmann and Kloner 2003).

Because skeletal muscle is easily obtained, is capable of regeneration and contains muscle precursor cells (myoblasts) that proliferate in culture, researchers are attempting to use this tissue as a source of cells for cardiac repair (Hassink, Brutel de la Riviere et al. 2003).

MSC can be cultured from non-hematopoietic bone marrow stromal cells and can differentiate into a cardiomyogenic cell type under appropriate culture conditions. Some early data suggest that allogeneic MSC may be less immunogenic than allogeneic hematopoietic progenitor cells (Orlic, Hill et al. 2002).

Myoblast Collection and Processing

Myoblast cultures are prepared from muscle biopsies, usually from the quadriceps muscle, which are minced and digested with enzymes and allowed to expand until the desired cell numbers are obtained. The predominant cell type in the resulting product consists of myoblasts, but other cell types such as fibroblasts are present in the cellular product, which is cryopreserved until administration (Pagani, DerSimonian et al. 2003).

Mesenchymal Stem Cell (MSC) Collection and Processing

MSC products are cultured from bone marrow, usually aspirated from the posterior iliac crest. After red blood cell removal and mononuclear cell enrichment, the cells are expanded in culture, harvested, pooled and cryopreserved (Hassink, Brutel de la Riviere et al. 2003). Cells from allogeneic donors may be stored as cell banks, aliquots of which may be used to prepare individual MSC products.

Unique Issues with Cellular Products

Products containing living cells cannot undergo sterilization procedures used for other drugs and biological products, therefore cellular products must be manufactured by methods that ensure sterility. Donors of source material can be tested for infectious diseases and, if there is adequate time before administration, cellular products can be tested for sterility. Due to inherent differences among individual donors, there can be large lot-to-lot inconsistencies even amongst products using the same manufacturing process. The FDA's approach in the review and regulation of cellular products for cardiac diseases has been similar to that employed for other cellular products regarding issues of donor testing, microbiological safety, and need for product characterization.

Microbiological Safety

For cells that are collected, processed, and dispensed in a period of less than 12 hours there is insufficient time to complete full microbial safety testing prior to patient administration. Those cellular products collected and processed in open or partially open systems are at the greatest risk for contamination with adventitious agents. These risks can be reduced by employing aseptic processing techniques. Although up to 14 days of incubation may be necessary to obtain a final sterility culture result, it is possible to obtain Gram stain and endotoxin results within approximately 2 hours. Therefore, these tests are required for product release and administration. It is possible for microbiological cultures to become positive days after the recipient has received the product. Consequently, FDA has requested that each proposed clinical study include a comprehensive action plan for physician and patient notification, patient monitoring (and treatment, if necessary), organism identification, antibiotic sensitivity, and investigation of contamination source should a positive culture of an infused product be reported.

Product Formulation

The composition of the administered cellular product depends on such factors as the cell source (bone marrow, peripheral blood, skeletal muscle), processing methods (red blood cell depletion, density gradient separation, CD34+ selection, culture) and storage conditions (short term at room temperature or 4°C, long term at 37°C, extended frozen storage). Additional data are needed to determine the effects of different formulation and storage conditions on the cellular product.

Product Characterization

The products described above consist of a heterogeneous population of cells that are not well characterized. Investigations of cellular products for cardiac diseases should explore methods of identifying and quantifying the cell populations that comprise the product. *In vitro* analysis of such features as morphology, viability, expression of phenotypic markers, proliferation and colony growth in culture, production of cytokines and other proteins, and gene expression can help determine which cells in a heterogeneous population may have therapeutic and deleterious actions. It may eventually become possible to correlate safety and efficacy with certain *in vitro* product characteristics. If these characteristics can be detected and quantified using available and reproducible assays, product specifications can be developed and utilized for release criteria.

Manufacturing Questions

Cellular products for treatment of cardiac disease may be obtained from bone marrow, peripheral blood or skeletal muscle of autologous or allogeneic donors. The products may be administered without manipulation or may be subjected to one or more selection, purification, cryopreservation or culture procedures. Because the specific cells, mechanisms of action and cell-device interactions are still in the early stages of investigation, the appropriate and adequate safety testing and characterization have not yet been defined and may vary based on the cell source and type of manipulation.

1. Please discuss the different intrinsic safety concerns for cellular products for the treatment of cardiac injury, and the testing that should be performed to ensure administration of a safe product, with consideration of the following variables:
 - a. Tissue source (bone marrow, peripheral blood, muscle)
 - b. Type and degree of product manipulation (cell isolation, cell selection, culture, expansion)
 - c. Final formulation (buffers, excipients, cell concentration)
 - d. Storage conditions (time, temperature)
 - e. Route and site of administration

2. Please comment on the elements of product identity and characterization necessary to generate data demonstrating safety and efficacy. Please consider the following:

- a. The degree of heterogeneity present in administered cellular products appears to be an important variable. Are there specific biomarkers that can identify cell types involved in cardiac repair? Are there specific biomarkers that can identify contaminating or damaged cells that may lead to adverse events when introduced into myocardial tissue?
- b. Based on the current state of knowledge, are there safety issues the agency should consider in relation to the type and relative percentage of cell types that can be identified by biomarkers including phenotype and/or other *in vitro* indicators in cellular products for cardiac repair? For example, can the relative percentages of fibroblasts in myoblast products or T-cells in stem cell products affect product safety or interfere with product performance?
- c. What other parameters could be assessed to further characterize these products for safety and potency?

PRECLINICAL STUDIES

Preclinical data derived from *in vivo* animal models supports the safety and suggests potential benefits of innovative therapies. Studies performed in animal models of disease provide insight regarding dose/activity and dose/toxicity relationships. Cellular products are complex and preclude a standard design of preclinical studies, as manufacturers might use in the development of drugs. The major sources of a cellular product's complexity include: the inherent biological heterogeneity of cellular products (in terms of both phenotypic and functional characteristics), potential safety concerns posed by novel routes of administration, cell-device interactions, and the effects of an immune response to the product.

Standard animal models of disease are frequently modified to generate the preclinical toxicity data needed for regulatory decisions. For example, immunological reactions to human cellular products in animal models often require that preclinical toxicology studies be performed with autologous animal cellular products, animal products that are analogous to the intended clinical product, rather than the actual human product. This approach is similar to an approach that is frequently used during preclinical testing of monoclonal antibodies directed against epitopes expressed only in humans, a situation in which an immune response or lack of applicable epitope limit the relevancy of the clinical product in the preclinical model.

In addition to providing toxicity data, preclinical studies may provide useful data regarding a cellular product's mechanism of action. In clinical studies, the distinction between pharmacologic and toxicologic effects is based, in part, upon an understanding of the mechanism(s) of action of the investigational product. The need to have an understanding of the biological actions of the investigational product can be a difficult criterion to meet. These studies are frequently based on hypotheses that are supported largely by *in vitro* data, limited animal studies, and/or anecdotal clinical experience, as is the case for many cellular products proposed for the treatment of cardiac diseases.

Cellular Products Manufactured Without *in vitro* Culture Methodology

Bone marrow-derived (BMSC) and/or blood (PBSC) cellular products, range from unmanipulated bone marrow cells to selected peripheral blood cell subpopulations enriched in cells expressing cell surface markers of stem cells such as CD34. The biology of these differing phenotypic subpopulations in cardiac tissue is not well understood. Hence, data derived from studies on one BMSC or PBSC product may or may not directly support the use of another cellular product.

Many questions remain about the safety and mechanisms of action of BMSC and PBSC for the treatment of cardiac diseases. Hypothesized mechanisms of action for BMSC and PBSC to explain improvement in cardiac function observed in some animal models include, but are not limited to: transdifferentiation into cardiac myocytes, neoangiogenesis, and inhibition of ventricular remodeling (Gehling, Ergun et al. 2000; Kocher, Schuster et al. 2001; Beltrami, Barlucchi et al. 2003; Orlic, Kajstura et al. 2003). Angioblasts contained in BMSC/PBSC products have been postulated to contribute to improvement of cardiac function by increasing perfusion of previously ischemic myocardium. BMSC/PBSC may also transdifferentiate into functional cardiac myocytes. If transdifferentiation occurs, the resulting cardiocytes may be abnormal and become arrhythmogenic, as suggested by a recent study (Zhang, Hartzell et al. 2002). The data suggest that the *in vivo* presence of the cells could be arrhythmogenic via any of the three classic mechanisms of arrhythmia: reentry, automaticity, or triggered activity.

There has been only one published preclinical study directly comparing skeletal myoblast and bone marrow-derived cellular products. These data suggest that both cellular products tested provided equivalent improvements in cardiac function, although FDA is not aware of these data having been replicated (Thompson, Emani et al. 2003).

Cellular Products Manufactured With *in vitro* Culture Methodology

Cellular products cultured from skeletal muscle biopsies contain differing proportions of fibroblasts and myocyte/myoblasts, as identified by immunophenotype and/or morphology. The relative percentage of these two cell types varies with the initial cell source and the subsequent manufacturing processes. The numbers of these major cell types may be an important factor in product development because they have different biological activities including, electrical excitability, contractility, and gene expression. Consequently, the cellular heterogeneity of a cellular product may pose unique safety concerns. For example, deleterious ventricular remodeling after an ischemic injury primarily results from fibroblast hypertrophy and proliferation. Ventricular remodeling could potentially be exacerbated by implantation of a cellular product containing predominantly fibroblasts, leading to adverse clinical outcomes.

Studies with transplantation of fetal cardiac cells into various animal species in the early 1990's showed that these cells can survive and function after transplant into normal cardiac microenvironments (Marelli, Desrosiers et al. 1992; Koh, Soonpaa et al. 1993). The initial demonstration that a cellular product derived from skeletal muscle biopsy could improve an animal's regional cardiac function came in 1998 in a rabbit model of myocardial injury produced by direct application of a cryoprobe (Taylor, Atkins et al. 1998). The method of injury did not produce ischemic damage comparable to what is

observed clinically, and only regional cardiac function was re-established. Subsequently, studies of ischemic cardiac disease in pigs demonstrated that cellular products derived from skeletal muscle biopsies could also improve the overall left ventricular ejection fraction (LVEF) (Jain, DerSimonian et al. 2001; Dib, Diethrich et al. 2002).

During the last decade, almost 50 published reports examined the engraftment of cultured, autologous cellular products derived from skeletal muscle biopsies into many animal species (mouse, rat, rabbit, pig, sheep) (Taylor, Atkins et al. 1998; Pouzet, Vilquin et al. 2000; Reinecke and Murry 2000; Scorsin, Hagege et al. 2000; Jain, DerSimonian et al. 2001; Suzuki, Brand et al. 2001; Chachques, Cattadori et al. 2002; Dengler and Katus 2002; Dib, Diethrich et al. 2002; Ghostine, Carrion et al. 2002; Leobon, Garcin et al. 2003). These data suggest that autologous cellular products derived from skeletal muscle biopsies can survive, engraft, and differentiate into striated muscle cells in both normal myocardium and myocardium injured by ischemia or toxins. Improved cardiac function has been reported based upon changes in one or more of the following: *in vitro* assessment of ventricular pressure (dP/dt) or force transduction; *in vivo* techniques of sonomicrometry or echocardiography. Dog and pig models are especially useful in assessing cardiac function because these models can be manipulated to produce acute, subacute, and chronic myocardial ischemia (Unger 2001).

Numerous unanswered questions remain regarding cellular products derived from skeletal muscle biopsies. Unlike cardiac muscle, skeletal muscle lacks intercalated disks and gap junctions (as evidenced by connexin-43 expression) (Suzuki, Brand et al. 2001). These structures allow normal myocardium to act as a syncytium for the efficient pumping action of the heart. The bulk of evidence from animal studies suggests that cellular products derived from skeletal muscle biopsies implanted into myocardium differentiate to form skeletal muscle that does not become electromechanically coupled to the native myocardium (Reffellmann and Kloner 2003). Therefore, although implanted skeletal muscle cells may contract, they do not become fully integrated into the heart muscle, resulting in a potentially arrhythmogenic focus. Data obtained from clinical studies, as well as from animal models, suggest that clinically significant arrhythmias are an important safety issue (Leobon, Garcin et al. 2003; Menasche, Hagege et al. 2003). Additional animal studies may be needed to explore the potential factors contributing to arrhythmogenesis such as:

- 1) the specific composition of the cellular product,
- 2) the dose of cells (absolute cell number and volume administered), and
- 3) the site of cell implantation (with respect to anatomic features such as major conduction pathways or valves and to location within a scarred, ischemic area of myocardium).

An alternative mechanism of action that has been suggested to explain the improvement in cardiac function observed in some animal studies of cellular products derived from skeletal muscle biopsies is a potential inhibitory effect of the cellular implants on ventricular remodeling. Implantation of the cellular product into an area of infarcted/ischemic myocardium may inhibit ventricular remodeling, an inhibition which may result in improved LVEF (Reffellmann and Kloner 2003). Although an appealing

hypothesis, it is clear that additional animal studies are needed to further explore this hypothesis.

Preclinical Summary

The adequacy of preclinical data supporting the safety of product administration to humans is fundamental to the design of early phase clinical trials. These preclinical data should be obtained from the use of the intended clinical cellular product (or an appropriate analogous product) delivered by the clinically relevant route of administration, using the clinically relevant delivery system, in an animal model that reflects the disease state of the patient population. Since cellular products have inherent cellular variability, the preclinical data may provide an important safety assessment of a cellular product prior to its use in clinical studies.

Preclinical Questions:

1. Various animal models have been proposed to support the safety of cellular products used in the treatment of cardiac disease. These include studies of both small (e.g., mouse, rat, rabbit) and large (e.g., dog, pig) species and studies utilizing either immune competent or immunocompromised animals. Each model provides distinct advantages and limitations. For instance, human cellular products can be tested in genetically immunocompromised rodents, but these animals provide limited clinical monitoring of cardiac function, and cannot be used to assess the safety of the devices used to administer the cells as proposed in the clinical studies. Large animal models allow for more extensive clinical monitoring of cardiac function and the use of the same delivery device intended for clinical use. However, use of immune competent species eliminates the ability to evaluate the safety of administration of the human cellular product.

Please discuss the merits and limitations of various large and small animal species for providing pharmacologic, physiologic, and toxicologic support for cellular products used in the treatment of cardiac diseases.

2. A central tenet of preclinical animal safety testing is that the test agent must possess biological activity in the animal model in order to provide meaningful data on both safety and activity endpoints. For cellular products, this tenet often necessitates using an analogous product in animal models in order to preserve biological activity. In particular, preclinical evaluation of cellular products for ischemic heart disease often employ animal models of acute ischemic heart disease (aortic constrictor, embolism, etc.), which can be used to generate safety data to support clinical trials. Specific issues that potentially can be addressed in animal models of disease include, but are not limited to, overall extent and duration of the effect of different doses of the injected cells on cardiac function and the effect of the route of administration and cell placement location on physiologic and safety outcomes.

Please discuss the merits of animal models of ischemic disease with respect to the ability to generate proof of concept (physiologic) data and to generate toxicologic data of relevance to the clinical disease.

INVESTIGATIONAL CATHETERS FOR DELIVERY OF CELLULAR PRODUCTS TO THE HEART

Percutaneous cardiac catheterization methods and devices are being actively investigated as a means to deliver cellular products. Current research in this area is focused primarily on development of cardiac catheters and methods that can provide targeted delivery of high concentrations of cell suspensions to specific regions of the myocardium. For example, a region of reversible myocardial ischemia previously identified by nuclear scan might be treated with an investigational cellular product either by catheter delivery into the coronary artery that supplies that region or by multiple injections into the same region of myocardium using a catheter that includes an injection needle at the distal end. Although bone marrow transplants have demonstrated that systemic, intravascular injection can successfully deliver therapeutic cell suspensions to some target organs, neither systemic delivery of cells nor treatment of the entire heart is a primary focus of current clinical research into delivery of cellular products for cardiac disease.

The earliest clinical reports of administration of cellular products for cardiac diseases primarily used direct, syringe-and-needle injection of cellular products through the exposed epicardial surface into the subjacent myocardium during concomitant thoracic surgery (Hamano, Nishida et al. 2001; Herreros, Prosper et al. 2003; Menasche, Hagege et al. 2003; Pagani, DerSimonian et al. 2003; Stamm, Westphal et al. 2003; Tse, Kwong et al. 2003). Although these studies demonstrated the feasibility of this delivery method, the risks of this invasive method are likely to preclude widespread use. The concept of percutaneous cardiac catheterization has proven to be widely applicable as a means to provide less invasive delivery of cardiac therapies that could initially be delivered only via surgery. Thus, there is interest in developing catheter-based methods for targeted delivery of cellular products to the myocardium. Recent clinical studies have largely reported the feasibility of two concepts for catheter delivery of these products: 1) infusion of cell suspensions into the coronary vasculature that supplies the target region of myocardium, and 2) injection of cell suspensions directly into the target region of myocardium using catheters that contain injection needles. Other concepts for catheter delivery of cellular therapies may also be feasible.

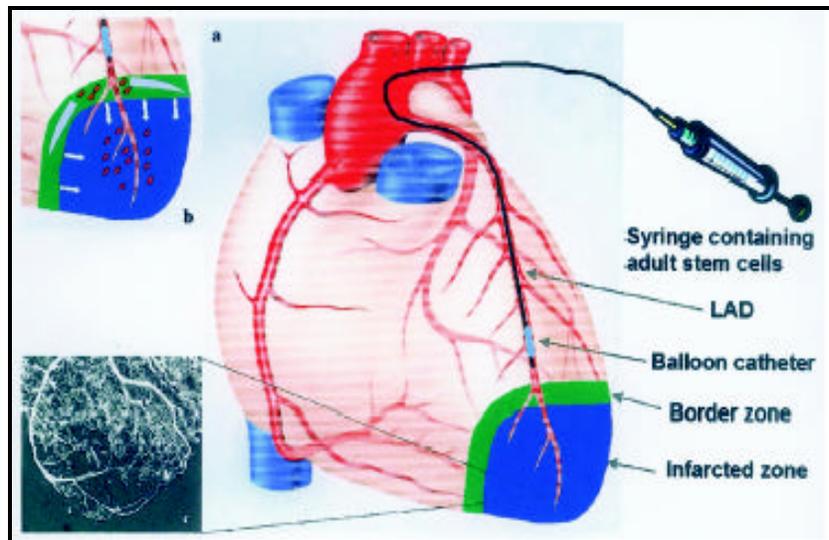
Infusion of Cellular Products into Coronary Arteries:

Preliminary studies have evaluated infusion of cell suspensions into individual coronary arteries using infusion pressures that exceed coronary artery pressure, a procedure that is relatively easy to perform. This method presumably relies upon migration of cells from the vasculature into the myocardium, but has the potential for causing coronary artery embolization. Therefore, this method may not be useful or feasible for delivery of all types of cellular products.

Clinical applications of this coronary artery infusion approach have used balloon catheters to occlude the coronary artery proximal to the desired treatment region, permitting infusion of cell suspensions at pressures that exceed coronary artery pressure. Delivery using elevated pressures is hypothesized to increase dispersion of the cell suspension within the vasculature of the affected region of myocardium and to

also increase adhesion and potential transmigration of the infused cells through the vascular endothelium. Following balloon inflation, a lumen within the balloon catheter or within a simple infusion catheter placed lateral to the balloon (i.e., between the balloon and the inner wall of the artery) is then used to infuse the cell suspension into the artery distal to the balloon. The infusion of a cell suspension into the coronary artery may be intentionally interrupted one or more times during the infusion process to permit balloon deflation and perfusion of the treated region by arterial blood. Standard methods for percutaneous catheterization of the coronary arteries are utilized, i.e., percutaneous insertion of a catheter into a large artery, often the femoral artery, such that the catheter may be directed retrograde through the aortic arch, then into the coronary arteries and then to the desired coronary artery location. Figure 1 below, copied from a recent publication, illustrates use of this method to infuse a cell suspension into an infarcted region of myocardium that is supplied by the anterior descending branch of the left coronary artery (Strauer, Brehm et al. 2002). In the illustration, the cell suspension is being infused distal to the inflated balloon.

Figure 1. Coronary Artery Infusion Of Cell Suspension



Small clinical case series have reported the feasibility of coronary artery infusion of cell suspensions when delivered within either hours or days following acute myocardial infarction (Assmus, Schachinger et al. 2002; Strauer, Brehm et al. 2002). More recently, abstracts have also reported use of this method to deliver cell therapies to cardiac regions affected by chronic myocardial infarction and ischemia. Note that placement of a coronary artery stent is increasingly used as a primary treatment for acute myocardial infarction (Aversano, Aversano et al. 2002; Andersen, Nielsen et al. 2003; Keeley, Boura et al. 2003). When balloon catheters are used to infuse cellular products soon after an acute myocardial infarction, the balloon can often be inflated within a recently deployed stent, thus reducing concerns regarding potential balloon injury to the arterial wall (Assmus, Schachinger et al. 2002; Strauer, Brehm et al. 2002).

There have been no reports of infusion of cellular products into coronary arteries producing undesirable embolic effects. However, a recently reported animal study that delivered a cell suspension to the coronary arteries of healthy canines produced acute myocardial ischemia followed by subacute microinfarction and fibrosis (Vulliet, Greeley et al. 2004).

Current case reports of this coronary artery infusion method have used coronary artery balloon catheters originally designed for other intended uses; no coronary artery catheter designed for delivery of cell suspensions distal to an occlusion balloon is currently approved for marketing in the U.S. Investigators have instead used coronary artery balloon angioplasty catheters that are designed to enlarge regions of fibrotic occlusion within the coronary arteries by stretching or "tearing" the occluded arterial segment as required. These catheters are designed to deliver relatively high pressures to the luminal surface of the artery and to expand the artery lumen to a specific diameter selected by the treating physician. The same balloon catheters are used to expand coronary artery stents within regions of occlusion. The diameter of these catheters is typically limited to approximately 1 mm such that they may be easily passed into the coronary arteries.

As is typically true for investigational therapies, we currently have an incomplete understanding of the medical-device-related safety and effectiveness issues associated with delivery of cell suspensions by coronary artery balloon catheters. Although many issues will be similar or identical to the issues encountered when these catheters are used for their intended use, other device-related issues will be specific to this new application.

- ?? One device concern relates to development and validation of methods for using a specific balloon catheter design to safely and effectively occlude a coronary artery without damaging the artery. Balloon angioplasty catheters are designed to selectively "damage" a coronary artery by stretching fibrotic, stenotic segments to a specific, larger diameter. Arterial stretch produced by therapeutic balloon angioplasty may induce rapid, arterial stenosis/restenosis by mechanisms of external arterial constriction (negative remodeling) and growth of new scar tissue on the luminal surface of the artery (intimal hyperplasia) (Heras, Chesebro et al. 1989; Schwartz, Murphy et al. 1991; Post, Borst et al. 1994; Serruys, de Jaegere et al. 1994; Mintz, Popma et al. 1996). This does not preclude the use of balloon angioplasty catheters for non-damaging arterial occlusion. Investigators may need to develop safe and effective methods for using the balloon catheters for non-damaging arterial occlusion. Animal studies may be indicated for development and validation of safe methods for use of a given model of balloon angioplasty catheter. The potential concern regarding balloon injury to the arterial wall may be lessened when a balloon angioplasty catheter is deployed within a previously expanded coronary artery stent.
- ?? A second device concern is that infusion of concentrated cell suspensions through a small-diameter catheter may create pressures high enough to rupture catheter materials or joints not designed or tested to sustain such pressures. The central lumen of a balloon angioplasty catheter is intended for passage of a small diameter guidewire. Neither the guidewire lumen nor the attached valves, connectors and tubing used for delivery of the guidewire and for flushing the lumen with saline

solution may have been designed or tested to sustain the pressures induced by infusion of concentrated cell suspensions. It may be important to test combinations of specific models of catheters and the intended cell suspensions prior to their use in early phase clinical trials.

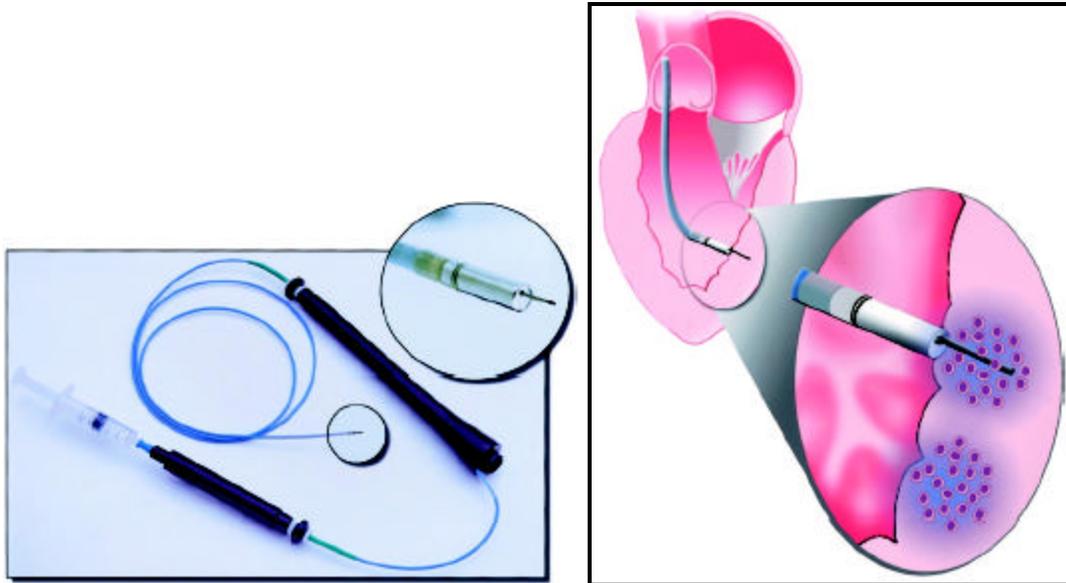
- ?? A third device concern is the possibility that contact with catheter materials may adversely affect the viability or functionality of the delivered cellular product. Cells contact the guidewire lumen plus attached valves, connectors and tubing. Additionally, guidewire lumens are commonly coated with lubricants designed to facilitate passage of the guidewire. FDA is not aware of published studies that have specifically examined this issue. However, a recent animal study that examined delivery of gene therapy via injection of viral vectors using a transvenous, intramyocardial needle injection catheter found that catheter lumen material strongly affected the transfection rate of the viral vectors (Naimark, Lepore et al. 2003).
- ?? A fourth device concern is the clogging of the long, small-diameter catheter lumen by concentrated cell suspensions.

Intramyocardial Injection of Cell Suspensions through Cardiac Catheters:

A second method for catheter delivery of cellular products to the myocardium is intramyocardial injection using either cardiac catheters or systems of catheters plus sheaths that include a retractable injection needle at the distal end. The injection needle is used to deliver multiple injections of a cell suspension into the targeted region of the myocardium. Clinical reports have been published in which catheters with needles were used for intramyocardial injection of cell suspensions into the subjacent myocardium (Fuchs, Satler et al. 2003; Perin, Dohmann et al. 2003; Smits, van Geuns et al. 2003).

Delivery of an injection catheter into the left ventricle requires percutaneous insertion of the catheters into a large artery, followed by retrograde passage of the catheter around the aortic arch, through the aortic valve, then into the left ventricle. Catheters or systems of catheters plus sheaths that are used for this purpose must also include the ability to control deflection of the catheter tip (or sheath tip) such that the catheter tip can be directed to the desired injection sites on the endocardial surface of the left ventricle. Unlike balloon angioplasty catheters that require only minimal shaft stiffness for effective use, injection catheters or systems of catheters plus sheaths must be sufficiently rigid to permit effective maintenance of contact with the moving ventricular wall of a contracting heart, while at the same time not being so excessively rigid that they pose an excessive risk of vascular or cardiac perforation during insertion or use. Figure 2, copied from a recent publication, illustrates the use of one investigational injection catheter that has been delivered through the aorta and across the aortic valve and that is being used to deliver multiple injections of a cell suspension into the left ventricular myocardium (Perin, Dohmann et al. 2003). The catheter illustrated below incorporates a catheter-tip deflection mechanism with a control on the catheter handle and an extensible-retractable injection needle that may be retracted back into the catheter following each injection. (The catheter tip within the heart is deflected in this illustration.)

Figure 2. Intramyocardial Injection Of Cell Suspension



No intramyocardial injection catheters are currently approved for marketing in the U.S.A. Other cardiac catheters such as radiofrequency cardiac ablation catheters and endocardial biopsy catheters are designed to controllably press the tip of the catheter against the endocardial surface of the heart at specific locations. Using these alternative catheters as models, suggests that deflectable, needle-tipped, intramyocardial injection catheters will be approximately 2 mm in diameter and that catheter and sheath systems that employ a deflectable sheath will be approximately 3 mm in diameter.

Device-related issues will be specific to intramyocardial injection catheters include the following:

- ?? Excessive needle extension may dispose a catheter to injection of cellular products completely through the myocardium into the pericardial or thoracic spaces or to creating injection needle damage in surrounding organs. Thus, catheters designed for this application should provide accurate, precise control of needle extension distance and should incorporate effective means to limit maximum needle extension distance. Tests for maximum needle extension under varying degrees of catheter tip deflection and simulating the 180° curve of the catheter around the aortic arch may be necessary.
- ?? A related concern is that animal studies suggest that, even with minimal needle extension, occasionally injecting cell suspensions may be injected through the myocardium and into the pericardial space. It may be important to consider whether cell suspensions pose a safety concern if they are injected into the pericardium or into the thoracic cavity or if they enter the systemic circulation (e.g., via lymphatic drainage of the pericardial sac).

- ?? Another concern is that some injections may be made into the left ventricular cavity, i.e., into the systemic circulation. Even with optimal technique, it may be difficult or impossible to maintain constant, stable contact between the catheter tip and the endocardial surface of the left ventricle during ventricular contraction, and so cell suspensions may inadvertently be injected into the left ventricular cavity. An animal study evaluating the actual stability of contact between the tip of a cardiac ablation catheter and the endocardial surface suggested that only 44% of “optimally stable” catheter placements, as judged by experienced electrophysiologists, were actually stable (movement < 2 mm) (Kalman, Fitzpatrick et al. 1997). It may be important to consider whether cell suspensions injected into the systemic circulation pose a safety concern.
- ?? Clogging of the injection lumen by concentrated cell suspensions may be a particularly important issue for intramyocardial injection catheters. For a variety of reasons, these catheters may use very small diameter needles. It may be desirable to deliver very small volumes of highly concentrated cell suspensions, in order to limit tissue trauma and inflammation. Small lumen diameters plus highly concentrated cell suspensions may increase the probability of clogging of the injection lumen. Prior to initiating early phase clinical studies, it may be necessary to determine whether the intended cell suspension can be delivered for the planned number of injections through the specific intramyocardial injection catheter without clogging.
- ?? Another issue is that catheter lumen materials used in intramyocardial injection catheters may adversely affect both viability and functionality of cell therapy suspensions. Note that, because it may be desirable to deliver only small volumes of cell suspension with these catheters it may be necessary to “fill” the catheter with cell suspension prior to insertion into the patient. This would increase the residence time of the cell suspension within the catheter lumen, increasing the interaction between the lumen materials and the cellular product.
- ?? Needle injection catheters or systems of catheters are a new type of device. Animal studies may be necessary to evaluate whether these devices cause excessive damage in the great vessels, the aortic valve, or intracardiac structures.
- ?? The injection depth and the “spread” of a cell suspension injection may affect the potential therapeutic effect. For example, injection into “more ischemic” locations near the endocardial surface of the heart may not produce the same effect as injection closer to the epicardial surface. Therefore, the therapeutic effect produced by the delivery of a particular cellular product through a particular injection catheter may not be reproduced if a different injection catheter is used because of the specific interaction between the catheter and the cellular product. Factors such as injection depth and spread and injection “success rate” may be influenced by catheter design, by the viscosity and volume of the injected cell suspension, etc. Unless the interaction between a specific intramyocardial injection catheter and a specific cellular product are proven to be unimportant, it may be necessary to perform animal testing to evaluate the effects produced by injecting a specific cellular product through a specific injection catheter.

Many novel combinations of delivery systems and cellular products are currently being evaluated. It is anticipated that additional delivery devices to deliver cellular products for cardiac diseases will be proposed by investigators. Types of delivery devices that have been proposed to date include: transepical administration via syringe and needle, transendocardial administration via needle injection catheters, and pressurized intravascular infusion into coronary arteries or veins that may be occluded via a balloon catheter.

Device Question:

1. Please provide recommendations regarding strategies for the use of animal models to evaluate the performance and safety of these delivery approaches including, but not limited to, comments on the specific points below.
 - a. Adverse effects on viability and function of the components of heterogeneous cellular product due to the extended exposure to metals (such as nitinol or stainless steel) and polymers.
 - b. Direct injection of cellular products into the myocardium usually requires delivery of small volumes of highly concentrated product. This may increase the likelihood of catheter obstruction. Please comment on factors, in addition to "simple" viscosity and cell concentration, that may contribute to this phenomenon.
 - c. Endovascular injection of cellular products into the myocardium may inadvertently lead to injection into the pericardial space, thoracic space, or systemic circulation. Please discuss ways to prevent unintentional injections into these sites.
 - d. To what extent are you concerned that depth of injection and spread of the injected cell suspension within the myocardium affect physiologic activity? How should these factors be evaluated in preclinical models of ischemic heart disease?

CLINICAL STUDIES

To illustrate the nature and extent of clinical studies being performed in order to assess the safety and bioactivity of cellular products in the treatment of cardiac disease, this section will summarize the major findings from certain publications describing the use of two tissue sources of cellular products: bone marrow and skeletal muscle.

Cellular Products Derived from Bone Marrow:

Overview:

As of early 2004, at least seven published clinical reports cite the use of bone marrow cells as a potential therapy for cardiac diseases. The indications have included acute myocardial infarction as well as chronic angina due to left ventricular ischemia. As discussed in prior sections, the cells administered in these studies were a mixture of

many different types of hematopoietic cells. All seven reports were from small sample-size, exploratory clinical studies that used an open label, non-randomized design. Clinical findings from the reports were notable for the absence of major safety concerns. However, the frequency, timing and types of safety assessments performed were not included in the publications. Similarly, the reports did not include data exploring the interactions between the cellular product administered and the delivery device used to administer the bone marrow.

Delivery Methods:

Three different methods were used to administer the bone marrow cellular product in these seven reports.

- ?? In two reports the cells were administered soon after an acute myocardial infarction was treated with stent placement in order to relieve the coronary artery obstruction (Assmus, Schachinger et al. 2002; Strauer, Brehm et al. 2002). A few days following the stenting procedure, a balloon catheter was placed at the site of the stent and inflated to occlude the coronary artery. The cellular product was then infused through the lumen of the balloon catheter over several two- to three- minute intervals with deflation of the balloon to allow coronary artery blood flow between administrations. One report noted that the cells were administered under “high pressure.”
- ?? In three of these reports, the bone marrow cells were administered transendocardially into subjects who had angina that was not amenable to conventional revascularization using either CABG or percutaneous coronary intervention (PCI) (Fuchs, Satler et al. 2003; Perin, Dohmann et al. 2003; Tse, Kwong et al. 2003). The region of the left ventricle to be injected was identified by scintigraphy as inadequately perfused. Catheterization was performed and that region was electroanatomically mapped (infarcted myocardium is electrically inert whereas viable myocardium supports an electrical potential) to select an area that was viable. An investigational injection catheter (Biosense Webster) was advanced into the left ventricle and placed in contact with the endocardium overlying the viable myocardium. The catheter’s 26-gauge needle was then extruded through the endocardium and the cells were injected into the subjacent myocardium. The process was repeated several times in the area identified as viable myocardium by electroanatomical mapping.
- ?? In two reports, the cellular products were administered transepically via a needle at the time of CABG (Hamano, Nishida et al. 2001; Stamm, Westphal et al. 2003). In one of the reports, the cells were injected into an area identified as ischemic but considered viable, (i.e. an area perfused by a stenotic coronary artery that was not amenable to CABG or PCI). In the other, the cells were injected directly into an area of a recent myocardial infarction, the target area also not suitable for CABG or PCI.

Outcomes:

In general, these seven clinical studies reported no clear improvement in clinical

outcomes in the treated subjects. However, all reports claimed to show improvement in some aspect of cardiac function. The significance of these improvements is difficult to evaluate because of the nature of the exploratory study designs. Several of the reports noted that subjects also received concomitant cardiac revascularization procedures, treatments which confound the assessment of the effects of the cell administration.

Table 1. Summary of Seven Published Reports of Cellular Products Derived from Bone Marrow

First Author	Assmus	Strauer	Perin	Fuchs	Tse	Stamm	Hamano
Indication	Acute MI	Acute MI	Severe ischemic LV dysfunction	Chronic angina	Chronic angina	Subacute MI, CABG	CABG, chronic angina
# of subjects	9	10	14	10	8	6	5
Delivery route	IC	IC	Endo	Endo	Endo	Epi	Epi
Delivery device(s)	Not published	Not published	Biosense injection catheter	Biosense injection catheter	Biosense injection catheter	22 gauge needle	26 gauge needle
# of cells	$245 \pm 72 \times 10^6$	$9-28 \times 10^6$	$25 \pm 6 \times 10^6$	$32 \pm 28 \times 10^6$	Not published	$1 - 3 \times 10^6$	$30 - 220 \times 10^6$
Concomitant procedure	Stenting during AMI	Stenting during AMI	None	None	None	CABG	CABG
Functional improvement	LVEF & local wall motion	Local wall motion	LVEF	Angina class	Angina class, local wall motion	LVEF, myocardial perfusion	Scintigraphic myocardial perfusion

LVEF = Left ventricular ejection fraction
 AMI = acute myocardial infarction
 IC = intracoronary
 Endo = transendocardial
 Epi = transepicardial

Cells Derived from Skeletal Muscle:

Overview:

As of early 2004, at least four published reports examine the use of skeletal muscle-derived cellular products in the treatment of cardiac disease. Additionally, the published literature includes a few case reports of use of these cellular products. The cells administered were thought to be predominantly myoblasts, but a variable fraction of the cells were probably other types, such as fibroblasts. All subjects in these reports had left ventricular systolic dysfunction due to previous myocardial infarction. All four published studies used uncontrolled study designs and each study enrolled a small number of

subjects (5 to 12). In two of the reports, the subjects underwent concomitant CABG and in one study, the cells were administered concomitant with implantation of a left ventricular assist device (LVAD) as a bridge to heart transplantation. These four publications did not reference any data exploring interactions between the cellular product and the delivery device.

Administration:

Of the four published reports:

- ?? In two of these reports, the cells cultured from muscle biopsies were administered transepicardially by a needle into the subjacent myocardium at the time of thoracotomy performed for CABG (Herrerros, Prosper et al. 2003; Menasche, Hagege et al. 2003). In both these reports, the cells were injected into an area of left ventricular infarction, as demonstrated by fluorine-18 fluorodeoxyglucose positron emission tomography. In one report, the coronary artery perfusing the area injected was not revascularized; in the other it was revascularized.
- ?? In one report, the cells were administered transepicardially by a needle at the time of thoracotomy performed for LVAD implantation (Smits, van Geuns et al. 2003). This study was performed to allow for histological examination of the heart at time of transplantation.
- ?? In the last report, the cells were administered transendocardially by an injection catheter into the subjacent myocardium (Pagani, DerSimonian et al. 2003). At catheterization that region was electroanatomically mapped (as described above) to select an area that was not viable. A Biosense Webster investigational injection catheter was advanced to the area and several injections were made.

Outcomes:

In all three non-LVAD reports, an improvement in wall thickening of the area was noted. Both CABG studies also demonstrated an improvement in global LVEF. The concomitant procedures, however, confound assessment of the effects of the cell administration. In three of four hearts explanted at time of transplantation in the LVAD report, a skeletal muscle-specific myosin heavy chain antibody identified mature myofibrils.

Notable safety findings in these four reports include the occurrence of arrhythmias. In one of the CABG reports (the one in which the area injected was not revascularized) two to four weeks after cell administration, four out of 10 subjects developed ventricular arrhythmias requiring defibrillator implantation. The other CABG study reported nonsustained ventricular tachycardia not requiring therapy 40 days after surgery. In the report in which the cells were injected transendocardially, one of five patients developed non-sustained ventricular tachycardia requiring defibrillator implantation 6 weeks after implantation. This report further states that out of another eight other subjects similarly treated, two died suddenly and three others had ventricular arrhythmias within three months of the procedure.

Given the small number of subjects in these reports, it is unclear if these ventricular arrhythmias were related to cell administration and no definitive association can be made between ventricular arrhythmias and number of cells administered, LVEF, or lack of revascularization of the area injected with cells. A recent editorial comment about one of these studies stated that, if related to cell administration, the ventricular arrhythmias may be due to “1) heterogeneity of action potentials between the native and the transplanted stem cells; 2) intrinsic arrhythmic potential of injected cells; 3) increased nerve sprouting induced by stem cell injection; and 4) local injury or edema induced by intramyocardial injection (Makkar, Lill et al. 2003).”

Table 2. Summary of Four Published Reports of Cellular Products Derived from Skeletal Muscle

First Author	Menasche	Herrerosa	Smits	Pagani
Indication	LVEF < 35% and scar due to MI	Scar due to MI & LVEF > 25%	NYHA class CHF > 1, LVEF 20 -45%	Listed for heart transplantation
# of subjects	10	12	5	5
Delivery route	Epi	Epi	Endo	Epi
Delivery device(s)	27-gauge needle	23-gauge needle	Biosense injection catheter	25 or 26-gauge needle
# of cells	500-1150 x 10 ⁶	0-393 x 10 ⁶	25-293 x 10 ⁶	300 x 10 ⁶ (first subject 2.2 x 10 ⁶)
Concomitant procedure	CABG (area injected not bypassed)	CABG (area injected bypassed)	None	LVAD
Improvement cited	LVEF & local wall thickening	LVEF & local wall thickening	LVEF	
Ventricular arrhythmias	4/10	1/12	1/5 & 5/8 (related studies)	None

LVAD = left ventricular assist device

Epi = transepicaldial

Endo = transendocardial

LVEF = left ventricular ejection fraction

Clinical Questions:

1. Please discuss the major types of adverse events you believe sponsors should focus upon during the follow-up evaluation of subjects receiving cardiac cellular

therapy products. Additionally, what frequency and duration of follow-up do you recommend? In addition to any other events, please consider the following potential adverse pathological and clinical events in your discussion items:

- a. Scar formation
 - b. Left ventricular dysfunction and congestive heart failure
 - c. Ventricular arrhythmias
 - d. Heart block
 - a. Neoplasia
2. Some adverse events potentially due to administration of these products, such as ventricular arrhythmias and worsening left ventricular contractility, may be identical to events that occur due to the natural history of the underlying disease. Consequently, adverse events related to the cellular product or its administration might not be discernable without concomitant controls. However, invasive procedures are frequently utilized to deliver these cellular products. Please discuss the pros and cons of using control groups in these early clinical studies, including any need for randomization or masking. Within your discussion, please also comment upon the use of placebos in the studies (e.g., transendocardial saline injection into the heart).

List of Questions:

Manufacturing:

Cellular products for treatment of cardiac disease may be obtained from bone marrow, peripheral blood or skeletal muscle of autologous or allogeneic donors. The products may be administered without manipulation or may be subjected to one or more selection, purification, cryopreservation or culture procedures. Because the specific cells, mechanisms of action and cell-device interactions are still in the early stages of investigation, the appropriate and adequate safety testing and characterization have not yet been defined and may vary based on the cell source and type of manipulation.

1. Please discuss the different intrinsic safety concerns for cellular products for the treatment of cardiac injury, and the testing that should be performed to ensure administration of a safe product, with consideration of the following variables:
 - a. Donor source (autologous or allogeneic)
 - b. Tissue source (bone marrow, peripheral blood, muscle)
 - c. Type and degree of product manipulation (cell isolation, cell selection, culture, expansion)
 - d. Final formulation (buffers, excipients, cell concentration)
 - e. Storage conditions (time, temperature)
 - f. Route and site of administration

2. Please comment on the elements of product identity and characterization necessary to generate data demonstrating safety and efficacy. Please consider the following:
 - a. The degree of heterogeneity present in administered cellular products appears to be an important variable. Are there specific biomarkers that can identify cell types involved in cardiac repair? Are there specific biomarkers that can identify contaminating or damaged cells that may lead to adverse events when introduced into myocardial tissue?

 - b. Based on the current state of knowledge, are there safety issues the agency should consider in relation to the type and relative percentage of cell types that can be identified by biomarkers including phenotype and/or other *in vitro* indicators in cellular products for cardiac repair? For example, can the relative percentages of fibroblasts in myoblast products or T-cells in stem cell products affect product safety or interfere with product performance?

 - c. What other parameters could be assessed to further characterize these products for safety and potency?

Preclinical:

3. Various animal models have been proposed to support the safety of cellular products used in the treatment of cardiac disease. These include studies of both

small (e.g., mouse, rat, rabbit) and large (e.g., dog, pig) species and studies utilizing either immune competent or immunocompromised animals. Each model provides distinct advantages and limitations. For instance, human cellular products can be tested in genetically immunocompromised rodents, but these animals provide limited clinical monitoring of cardiac function, and cannot be used to assess the safety of the devices used to administer the cells as proposed in the clinical studies. Large animal models allow for more extensive clinical monitoring of cardiac function and the use of the same delivery device intended for clinical use. However, use of immune competent species eliminates the ability to evaluate the safety of administration of the human cellular product.

Please discuss the potential benefits, along with the limitations of various large and small animal species for providing pharmacologic, physiologic, and toxicologic support for cellular products used in the treatment of cardiac diseases.

4. A central tenet of preclinical animal safety testing is that the test agent must possess biological activity in the animal model in order to provide meaningful data on both safety and activity endpoints. For cellular products, this tenet often necessitates using an analogous product in animal models in order to preserve biological activity. In particular, preclinical evaluation of cellular products for ischemic heart disease often employ animal models of acute ischemic heart disease (aortic constrictor, embolism, etc.), which can be used to generate safety data to support clinical trials. Specific issues that potentially can be addressed in animal models of disease include, but are not limited to, overall extent and duration of the effect of different doses of the injected cells on cardiac function and the effect of the route of administration and cell placement location on physiologic and safety outcomes.

Please discuss the merits of animal models of ischemic disease with respect to the ability to generate proof of concept (physiologic) data and to generate toxicologic data of relevance to the clinical disease.

Device:

5. Many novel combinations of delivery systems and cellular products are currently being evaluated. It is anticipated that additional delivery devices to deliver cellular products for cardiac diseases will be proposed by investigators. Types of delivery devices that have been proposed to date include: transepical administration via syringe and needle, transendocardial administration via needle injection catheters, and pressurized intravascular infusion into coronary arteries or veins that may be occluded via a balloon catheter.

Please provide recommendations regarding strategies for the use of animal models to evaluate the performance and safety of these delivery approaches including, but not limited to, comments on the specific points below.

- a. Adverse effects on viability and function of the components of heterogeneous cellular product due to the extended exposure to metals (such as nitinol or stainless steel) and polymers.
- b. Direct injection of cellular products into the myocardium usually requires delivery of small volumes of highly concentrated product. This may increase the likelihood of catheter obstruction. Please comment on factors, in addition to "simple" viscosity and cell concentration, that may contribute to this phenomenon.
- c. Endovascular injection of cellular products into the myocardium may inadvertently lead to injection into the pericardial space, thoracic space, or systemic circulation. Please discuss ways to prevent unintentional injections into these sites.
- d. To what extent are you concerned that depth of injection and spread of the injected cell suspension within the myocardium affect physiologic activity? How should these factors be evaluated in preclinical models of ischemic heart disease?

Clinical:

6. Please discuss the major types of adverse events you believe sponsors should focus upon during the follow-up evaluation of subjects receiving cardiac cellular therapy products. Additionally, what frequency and duration of follow-up do you recommend? In addition to any other events, please consider the following potential adverse pathological and clinical events in your discussion items:
 - a. Scar formation
 - b. Left ventricular dysfunction and congestive heart failure
 - c. Ventricular arrhythmias
 - d. Heart block
 - d. Neoplasia
7. Some adverse events potentially due to administration of these products, such as ventricular arrhythmias and worsening left ventricular contractility, may be identical to events that occur due to the natural history of the underlying disease. Consequently, adverse events related to the cellular product or its administration might not be discernible without concomitant controls. However, invasive procedures are frequently utilized to deliver these cellular products. Please discuss the pros and cons of using control groups in these early clinical studies, including any need for randomization or masking. Within your discussion, please also comment upon the use of placebos in the studies (e.g., transendocardial saline injection into the heart).

Attachments:

1. NIH internet web site: <http://stemcells.nih.gov/infoCenter/stemCellBasics.asp>. Stem cell basics.
2. Wognum, A, Eaves, A, Thomas, T. Identification and isolation of hematopoietic stem cells. *Arch Med Res* 2003;34:461-475.
3. Orlic, D, Hill, J, Arai, A. Stem cells for myocardial regeneration. *Circ Res* 2002;91:1092-1102.
4. Hassink, R, de la Riviere, et. al., Transplantation of cells for cardiac repair. *J Am Coll Cardiol* 2003;41:711-777.
5. Strauer, B, Brehm, M, et. al., Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 2002;106:1913-1918.
6. Perin, E, Dohmann, H, et. al., Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation* 2003;107:2294-2302.
7. Ghostine, S., et al., Long-term efficacy of myoblast transplantation on regional structure and function after myocardial infarction. *Circulation*, 2002. 106(12 Suppl 1): p. I131-6.
8. Kocher, A.A., et al., Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med*, 2001. 7(4): p. 430-6.
9. Unger, E.F., Experimental evaluation of coronary collateral development. *Cardiovasc Res*, 2001. 49:497-506.
10. Thompson, R.B., et al., Comparison of intracardiac cell transplantation: autologous skeletal myoblasts versus bone marrow cells. *Circulation*, 2003. 108 Suppl 1: p. II264-71.
11. Naimark, W, Lepore, J, et. al., Adenovirus-catheter compatibility increases gene expression after delivery to porcine myocardium. *Hum Gene Ther* 2003;14:161-166.
12. Korbling, M, Estrov, Z. Adult stem cells for tissue repair—a new therapeutic concept? *N Engl J Med* 2003;349:570-582.
13. Rosenthal, N. Prometheus's vulture and the stem-cell promise. *N Engl J Med* 2003;349:267-274.
14. Forrester, J, Price, M, Makkar, R. Stem cell repair of infarcted myocardium. *Circulation* 2003;108:1139-1145.

Cited References

- Andersen, H. R., T. T. Nielsen, et al. (2003). "A comparison of coronary angioplasty with fibrinolytic therapy in acute myocardial infarction." N Engl J Med **349**(8): 733-42.
- Assmus, B., V. Schachinger, et al. (2002). "Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI)." Circulation **106**(24): 3009-17.
- Aversano, T., L. T. Aversano, et al. (2002). "Thrombolytic therapy vs primary percutaneous coronary intervention for myocardial infarction in patients presenting to hospitals without on-site cardiac surgery: a randomized controlled trial." Jama **287**(15): 1943-51.
- Beltrami, A. P., L. Barlucchi, et al. (2003). "Adult cardiac stem cells are multipotent and support myocardial regeneration." Cell **114**(6): 763-76.
- Chachques, J. C., B. Cattadori, et al. (2002). "Treatment of heart failure with autologous skeletal myoblasts." Herz **27**(7): 570-8.
- Dengler, T. J. and H. A. Katus (2002). "Stem cell therapy for the infarcted heart ("cellular cardiomyoplasty")." Herz **27**(7): 598-610.
- Dib, N., E. B. Diethrich, et al. (2002). "Endoventricular transplantation of allogenic skeletal myoblasts in a porcine model of myocardial infarction." J Endovasc Ther **9**(3): 313-9.
- Fuchs, S., L. F. Satler, et al. (2003). "Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease: a feasibility study." J Am Coll Cardiol **41**(10): 1721-4.
- Gehling, U. M., S. Ergun, et al. (2000). "In vitro differentiation of endothelial cells from AC133-positive progenitor cells." Blood **95**(10): 3106-12.
- Ghostine, S., C. Carrion, et al. (2002). "Long-term efficacy of myoblast transplantation on regional structure and function after myocardial infarction." Circulation **106**(12 Suppl 1): I131-6.
- Gunsilius, E., G. Gastl, et al. (2001). "Hematopoietic stem cells." Biomed Pharmacother **55**(4): 186-94.
- Hamano, K., M. Nishida, et al. (2001). "Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: clinical trial and preliminary results." Jpn Circ J **65**(9): 845-7.
- Hassink, R. J., A. Brutel de la Riviere, et al. (2003). "Transplantation of cells for cardiac repair." J Am Coll Cardiol **41**(5): 711-7.

- Heras, M., J. H. Chesebro, et al. (1989). "Effects of thrombin inhibition on the development of acute platelet-thrombus deposition during angioplasty in pigs. Heparin versus recombinant hirudin, a specific thrombin inhibitor." Circulation **79**(3): 657-65.
- Herreros, J., F. Prosper, et al. (2003). "Autologous intramyocardial injection of cultured skeletal muscle-derived stem cells in patients with non-acute myocardial infarction." Eur Heart J. **24**(22): 2012-20.
- Jain, M., H. DerSimonian, et al. (2001). "Cell therapy attenuates deleterious ventricular remodeling and improves cardiac performance after myocardial infarction." Circulation **103**(14): 1920-7.
- Kalman, J. M., A. P. Fitzpatrick, et al. (1997). "Biophysical characteristics of radiofrequency lesion formation in vivo: dynamics of catheter tip-tissue contact evaluated by intracardiac echocardiography." Am Heart J **133**(1): 8-18.
- Keeley, E. C., J. A. Boura, et al. (2003). "Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomised trials." Lancet **361**(9351): 13-20.
- Kocher, A. A., M. D. Schuster, et al. (2001). "Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function." Nat Med **7**(4): 430-6.
- Koh, G. Y., M. H. Soonpaa, et al. (1993). "Long-term survival of AT-1 cardiomyocyte grafts in syngeneic myocardium." Am J Physiol **264**(5 Pt 2): H1727-33.
- Leobon, B., I. Garcin, et al. (2003). "Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host." Proc Natl Acad Sci U S A **100**(13): 7808-11.
- Makkar, R. R., M. Lill, et al. (2003). "Stem cell therapy for myocardial repair: is it arrhythmogenic?" J Am Coll Cardiol **42**(12): 2070-2.
- Marelli, D., C. Desrosiers, et al. (1992). "Cell transplantation for myocardial repair: an experimental approach." Cell Transplant **1**(6): 383-90.
- Menasche, P., A. A. Hagege, et al. (2003). "Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction." J Am Coll Cardiol **41**(7): 1078-83.
- Mintz, G. S., J. J. Popma, et al. (1996). "Arterial remodeling after coronary angioplasty: a serial intravascular ultrasound study." Circulation **94**(1): 35-43.
- Mukherjee, D., D. L. Bhatt, et al. (1999). "Direct myocardial revascularization and angiogenesis--how many patients might be eligible?" Am J Cardiol **84**(5): 598-600, A8.

- Naimark, W. A., J. J. Lepore, et al. (2003). "Adenovirus-catheter compatibility increases gene expression after delivery to porcine myocardium." Hum Gene Ther **14**(2): 161-6.
- Orlic, D., J. M. Hill, et al. (2002). "Stem cells for myocardial regeneration." Circ Res **91**(12): 1092-102.
- Orlic, D., J. Kajstura, et al. (2003). "Bone marrow stem cells regenerate infarcted myocardium." Pediatr Transplant **7 Suppl 3**: 86-8.
- Pagani, F. D., H. DerSimonian, et al. (2003). "Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. Histological analysis of cell survival and differentiation." J Am Coll Cardiol **41**(5): 879-88.
- Perin, E. C., H. F. Dohmann, et al. (2003). "Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure." Circulation **107**(18): 2294-302.
- Post, M. J., C. Borst, et al. (1994). "The relative importance of arterial remodeling compared with intimal hyperplasia in lumen renarrowing after balloon angioplasty. A study in the normal rabbit and the hypercholesterolemic Yucatan micropig." Circulation **89**(6): 2816-21.
- Pouzet, B., J. T. Vilquin, et al. (2000). "Intramyocardial transplantation of autologous myoblasts: can tissue processing be optimized?" Circulation **102**(19 Suppl 3): III210-5.
- Reffellmann, T. and R. A. Kloner (2003). "Cellular cardiomyoplasty--cardiomyocytes, skeletal myoblasts, or stem cells for regenerating myocardium and treatment of heart failure?" Cardiovasc Res **58**(2): 358-68.
- Reinecke, H. and C. E. Murry (2000). "Transmural replacement of myocardium after skeletal myoblast grafting into the heart. Too much of a good thing?" Cardiovasc Pathol **9**(6): 337-44.
- Schwartz, R. S., J. G. Murphy, et al. (1991). "Coronary artery restenosis and the "virginal membrane": smooth muscle cell proliferation and the intact internal elastic lamina." J Invasive Cardiol **3**: 3-8.
- Scorsin, M., A. Hagege, et al. (2000). "Comparison of the effects of fetal cardiomyocyte and skeletal myoblast transplantation on postinfarction left ventricular function." J Thorac Cardiovasc Surg **119**(6): 1169-75.
- Serruys, P. W., P. de Jaegere, et al. (1994). "A comparison of balloon-expandable-stent implantation with balloon angioplasty in patients with coronary artery disease. Benestent Study Group." N Engl J Med **331**(8): 489-95.
- Smits, P. C., R. J. van Geuns, et al. (2003). "Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: clinical experience with six-month follow-up." J Am Coll Cardiol **42**(12): 2063-9.

- Stamm, C., B. Westphal, et al. (2003). "Autologous bone-marrow stem-cell transplantation for myocardial regeneration." Lancet **361**(9351): 45-6.
- Strauer, B. E., M. Brehm, et al. (2002). "Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans." Circulation **106**(15): 1913-8.
- Suzuki, K., N. J. Brand, et al. (2001). "Overexpression of connexin 43 in skeletal myoblasts: Relevance to cell transplantation to the heart." J Thorac Cardiovasc Surg **122**(4): 759-66.
- Taylor, D. A., B. Z. Atkins, et al. (1998). "Regenerating functional myocardium: improved performance after skeletal myoblast transplantation." Nat Med **4**(8): 929-33.
- Thompson, R. B., S. M. Emani, et al. (2003). "Comparison of intracardiac cell transplantation: autologous skeletal myoblasts versus bone marrow cells." Circulation **108** **Suppl 1**: II264-71.
- Tse, H. F., Y. L. Kwong, et al. (2003). "Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation." Lancet **361**(9351): 47-9.
- Unger, E. F. (2001). "Experimental evaluation of coronary collateral development." Cardiovasc Res **49**(3): 497-506.
- Vulliet, P. R., M. Greeley, et al. (2004). "Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs." Lancet **363**: 783-84.
- Wognum, A. W., A. C. Eaves, et al. (2003). "Identification and isolation of hematopoietic stem cells." Arch Med Res **34**(6): 461-75.
- Yeh, E. T., S. Zhang, et al. (2003). "Transdifferentiation of human peripheral blood CD34+-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo." Circulation **108**(17): 2070-3.
- Zhang, Y. M., C. Hartzell, et al. (2002). "Stem cell-derived cardiomyocytes demonstrate arrhythmic potential." Circulation **106**(10): 1294-9.